

Synthesis of single-stranded RNA probe for mRNA detection in tissue sections

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Reagents

- ◆ 10 × transcription buffer: 400 mM Tris-HCl pH 7.5, 60 mM MgCl₂, 20 mM spermidine, 100 mM NaCl
- ◆ Nucleotide mix: 2.5 mM ATP, 2.5 mM GTP, and 2.5 mM UTP
- ◆ TE: 50 mM Tris-HCl, 1 mM EDTA pH 8—sterilize by autoclaving
- ◆ Phenol:chloroform: mix phenol, chloroform, isoamyl alcohol in the ratio 25:24:1—store at 4 °C

Method

This easy method gives good results but for a more precise hydrolysis to the correct length see [Synthesis of DIG or fluorescein labelled RNA probe](#)

1 Mix the following reagents at room temperature:

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|--|--------|
| • 10 × transcription buffer | 2 μl |
| • 100 mM dithiothreitol (DTT) | 2 μl |
| • recombinant RNasin ribonuclease inhibitor (20 U/μl) | 1 μl |
| • nucleotide mix | 4 μl |
| • 100 μM CTP | 2 μl |
| • linearized template DNA (1 μg/μl) | 1.5 μl |
| • [α - ³⁵ S]CTP (1000 Ci/mmol, 10 mCi/ml) | 6 μl |
| • RNA polymerase (20 U/μl) | 2.5 μl |

2 Incubate for 60–90 min at 37 °C.

3 Add RNase-free DNase at 1 U/μg of template DNA.

4 Incubate 30–45 min at 37 °C.

5 Add 300 μl of 1 M ammonium acetate and 20 μg of tRNA.

6 Add 2 vol. of phenol:chloroform, vortex, and spin for 2 min in a microcentrifuge.

- 7 Recover the aqueous phase and precipitate RNA probe by adding 2.5 vol. of 100% ethanol, mix, and chill for 10 min at -70°C . Spin for 5 min in a microcentrifuge.
- 8 Redissolve the pellet in 100 μl TE.
- 9 Load on a 1 ml Sephadex G50 spun column, centrifuge 10 min at 2000 r.p.m., and recover the eluate (Eppendorf centrifuge 5810R).
- 10 To partially hydrolyse the probe add 0.1 vol. of freshly prepared 1 M sodium hydroxide and keep on ice for 10–20 min.^a
- 11 Precipitate RNA by adding 20 μg tRNA, 0.1 vol. of 3 M sodium acetate pH 5.5, and 2.5 vol. of 100% ethanol, and chill for 10 min at -70°C . Spin in microcentrifuge for 5 min.
- 12 Redissolve the pellet in 100 μl of TE, mix 1 μl of probe in scintillation liquid, and determine the amount of radioactivity (c.p.m.).^b

Notes

- a To improve the penetration of probe into tissues, the length of RNA probe can be reduced to 0.1–0.25 kb (see Chapter 1 in [In Situ Hybridization 2e](#)).
- b A good probe yields between 8×10^5 and 1.5×10^6 c.p.m./ μl .